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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Hiroki FUKUI

Application/Control No. 10/091,992, filed: 31/03/1999

for: HIGH PURITY POLYSACCHARIDE CONTAINING HYDROPHOBIC  
GROUP AND PROCESS FOR PRODUCING IT

Honorable Commissioner of Patents and Trademarks  
United States Patent and Trademark Office  
Washington, D. C. 20231

Sir:

DECLARATION UNDER 37 CFR 1.132

I, Hiroki FUKUI, declare and state that:

1. I am one of inventors of the present invention.
2. Concerning the above-identified application, I  
carried out the following experiment.

Experiment

## Comparative Example 2

By the same procedures as in Example 1-2, a pullulan-cholesterol derivative was synthesized. After completion of the reaction, a superfluous amount of ethanol was introduced into the reaction liquor to cause formation of precipitate, which was purified in the following manner. Thus, the supernatant liquid was removed and ethanol was added to the remaining precipitate, whereupon the precipitate was collected by filtration and was subjected to vacuum drying. 0.5 g of the so-obtained white powder was taken out and was dissolved in 200 ml of pure water. The resulting aqueous solution was once ultrasonicated to disperse the contents therein uniformly, whereupon the solution was charged in a dialysis membrane (Spectra/Por 3: a product of the firm Spectrapor; exclusion molecular weight of 3,500) to subject to a dialysis against about 10 liters of pure water. The dialysis was continued for five days while exchanging pure water once a day. After the dialysis, the resulting liquid was subjected to a freeze-drying, whereby about 0.4 gram of white powdery product was obtained.

The above purified product was analyzed by <sup>1</sup>H-NMR as in Example 1-2 to determine the content of the cholesterol dimer. On the other hand, a sample before the ultrasonication and the purified product were analyzed by SEC in the same manner as in Example 1-3. The results were as shown in Table 9.

Table 9

|  |            |
|--|------------|
| Content of unsubstituted pullulan      | 3.6 wt. %  |
| Content of cholesterol dimer           | 0.5 wt. %  |
| Content of CHP in the purified product | 95.9 wt. % |

In Example 1-2, cholesterol dimer was removed completely by acetone-reprecipitation, whereas in Comparative Example 2 using ethanol-reprecipitation, a considerable amount of cholesterol dimer remained. In Example 1-3, unsubstituted pullulan was removed by ultracentrifugation, whereas in Comparative Example 2 using dialysis, removal thereof was failed.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

Date: \_\_\_\_\_

\_\_\_\_\_  
Hiroki FUKUI